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RESEARCH ARTICLE

Isolation and enumeration of bacteria from common green vegetables available in nearby market at Ayodhya

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Abstract
The present investigation was a part of the project work to identify the microbes present in the edible vegetables procured from the local markets at and around Ayodhya and Faizabad. Fresh vegetables were collected and brought to the laboratory of the Department of Microbiology, Dr Rammanohar Lohia Awadh University, Ayodhya. Standard procedures were adopted to isolate, identify and study the microflora obtained. A list of Gram-positive and Gram-negative bacteria is made on the basis of the findings of the present study.
Keywords: Gram-positive, Gram-negative, Microbes, Ayodhya.

Introduction
Vegetables are a vital part of every healthy diet and balanced meal due to their high nutritional value, which includes vitamins, minerals, and phytonutrients as well as their support of the body's metabolic processes (WHO 2003).

Fresh fruits and vegetables carry an abundance of bacteria on their surfaces, not all of which cause disease. Vegetables develop differently depending on various times of the year which is ideal for their growth conditions. Various sources can infect vegetables. Various sources, including soil, water, insects, and air, from birds, animals, and machinery used in agriculture advertising them. Vegetables typically have 104 to 107 or 103 to 105 microorganisms/cm^3 microorganisms/g. Several of the most common bacteria kinds include Corynebacterium, lactic acid bacteria, Proteus, Micrococcus, Enterococcus, and spore-formers and Pseudomonas.

Gram-positive and Gram-negative Bacteria
Because they change the functional properties of the food products and produce new flavors, aromas, or textures, some beneficial bacteria may be essential. Bacteria, mold, yeast, algae, viruses, parasitic worms, protozoa, and other microbes can all be found in food. These species' size, form, biochemistry, and cultural characteristics differ. The microorganisms listed below are some of the most significant genera and species frequently found in food products. The food and environmental requirements vary depending on the type of microorganism.

Acinetobacter sps.

The genus Acinetobacter belongs to the Gram-negative bacteria subclass Gamma proteo bacteria. Magnification reveals the oxidase-negative, non-motile, and typically found in pairs of *Acinetobacter* species. The morphologies of young cultures resemble rods. Since they are strong aerobes, they do not reduce nitrates. They are important creatures of the soil and water, and they are also found on a variety of foods, especially fresh items that have been refrigerated. Nosocomial pneumonia frequently results from *A. baumannii*, especially when the use of a ventilator is just getting started. Additionally, it may cause meningitis, bacteremia, skin infections, and open wounds.

Bacillus sps.

Gram-positive, aerobic, long, thick-rod-shaped, and catalase-positive, *B. cereus* is a key player in foodborne illness. It also forms spores. It typically dwells in the soil and

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is not close to a variety of food sources. Eating cooked and room temperature foods, such as desserts, meat, dinners, dairy products, rice, pasta, etc., frequently results in diarrheal sickness since it is thermo duric. Some B. cereus strains are psychrotrophic, meaning they grow at refrigerator temperature.

Salmonella sps.

Salmonella spp. are reportedly a significant contributor to human foodborne illnesses. The most typical foodborne bacterial illness in humans is salmonellosis. Typhoid and paratyphoid strains are associated with enteric fever, a serious sickness that affects humans. *Enterobacteriaceae, Salmonella*. The best development temperature is from 37 to 45°C. The organism can also grow in food at about 7°C. Carbohydrates are fermented, which results in the production of acid and gas. Salmonella exclusively use citrate as a carbon source, are catalase-positive, oxidase-negative, and produce H2S. While certain strains of *Salmonella* exhibit psychrotrophic traits, others can grow at temperatures as high as 54°C. The optimal pH range for the organism is between 6.5 and 7.5. However, it can grow from 4.5 to 9.5 for expansion spp. are small, gram-negative, nonspore forming, rod-shaped, facultative anaerobic bacteria. They are members of the family.

Shigella sps.

Shigellosis, or bacillary dysentery, is caused by *Shigella* species. *Shigella* belongs to the family Enterobacteriaceae. The growth temperature range is 10 to 48°C. Low pH meals are often not favorable for *Shigella* growth. For *Shigella*, ionising radiation is dangerous. In contrast to Salmonellae, species are non-motile, do not grow on KCN agar, do not grow on citrate as their primary carbon source, and only produce acid from carbohydrates. Shigellosis is a dangerous disease that can affect both industrialized and developing countries. Eating polluted food causes disease.

Escherichia sps.

Some *E. coli* strains have a connection to foodborne gastroenteritis. These gram-negative, lactose fermenting progeneous rods on Endo agar produce dark colonies with a metallic shine. The organism grows on a wide range of mediums and on a variety of diets. They can survive in a variety of pH and temperature conditions (4 to 46°C) (4.4 to 9.0). However, they grow very slowly when food is stored at refrigerator temperature (5°C). They belong to the family *Enterobacteriaceae*. The bacteria also acts as a faecal contamination indicator. The bacterium can also cause meals to taste odd and to create gas and acid.

Clostridium sps.

Clostridiun botulinum is the source of the known toxin with the highest lethality. It is a gram-positive, anaerobic rod-shaped bacteria. Oval endospores develop in stationary phase cultures. There are seven distinct types of *C. botulinum*, each with a unique neurotoxin produced based on the neurotoxin's serological specificity (A to G). A rare yet fatal sickness, botulism. A neurotoxin produced by the bacterium and consumed through food has the potential to be lethal. On the other hand, heat can quickly render the poison inactive (a protein).

Erwinia sps.

The bulk of the bacteria in the genus Erwinia are members of the *Enterobacteriaceae* plant pathogenic subfamily. The first phytobacteriologist, Erwin Smith, was the name of the organism. It is a gram-negative bacteria having ties to *Yersinia, Shigella, Salmonella,* and *E. coli*. It is, in essence, a rod-shaped bacterium. One well-known species in this genus is *E. amylovora*, which causes fire blight on apple, pear, and other Rosaceous crops. Numerous plant diseases are brought on by another species, *Erwinia carotovora* (also called *Pectobacterium carotovorum*). These species' pectolytic enzymes hydrolyze the pectin that connects individual plant cells. Bacterial soft rot is a common term used to describe degradation caused by *E. carotovora* (BSR). All except the most severely damaged plants and plant parts may resist bacterial invasion of any kind

Pediococcus sps.

The *pediococci* group of bacteria is commercially important to the food and brewing industries. The fermentation of fruits, vegetables, meats, sausage products, fermented milks, and the flavoring of Cheddar and other closely related cheese varieties have all used *pediococci* species and strains as starter cultures. Some strains make beer ropy and sticky by producing capsular material.

Some Vegetables and Nutritional Value

Tomatoes

Tomatoes are categorized as a vegetable even though, due to their uses, they are really a fruit. They were known as "apples of love" in French. Fresh New Zealand tomatoes are allowed to mature all year round in greenhouses.

Nutrition

Tomatoes are a good dietary supply of vitamin C and A and a significant amount of potassium (from beta-carotene). The carotenoid lycopene is the most well-known phytonutrient found in tomatoes and is responsible for their distinctive red colour. It is noteworthy since it is uncommon in other foods and is under investigation for a number of potential health benefits. Other carotenoids, in particular beta-carotene, are also present along with phenolic acids and flavonoids.

Cabbages

Cabbage comes in red, green, or white kinds with round or oval, smooth or crinkled leaves that are produced in India and many other nations. The subtle changes in taste between the different cabbage color varieties.

Nutrition

Cabbages (white/green) are a good source of vitamin C and vitamin K and a significant supply of potassium.

- Red cabbage is a good source of vitamin C, dietary fibre, vitamin A, and vitamins B6.
- There are differences between the types; for example, red cabbage contains a lot more vitamin C and B6 than green cabbage.
- Like other members of the brassica family, cabbage contains a number of phytonutrients, such as glucosinolates, carotenoids, and phenolic compounds.

Capsicum/Shimla Mirch

There are many distinct varieties of capsicum, and some are more suitable than others for particular uses. If you intend to eat them raw, choosing a kind with a soft skin is imperative. Numerous chiles ripen spontaneously and change colour, typically going from green to black or brown to red. The heat increases with the ripeness of the capsicum. Shi*m*L*a* mirch comes in a variety of varieties, some of which are more vegetable than others.

Nutrition

In addition to being a strong source of vitamin C, chilli peppers include a range of vitamins and minerals. Since most people only consume small amounts of chilli peppers, their flavour is more important than their nutritional value.

Pointed Gourd/Padwal/Parwal

It is frequently referred to as "green potato" in India. It is commonly grown in India's eastern region, particularly in Orissa, Bengal, Assam, Bihar, and Uttar Pradesh. It is a dioecious cordate vine (creeper), male and female plants, that is grown on a trellis.

Nutrition

Vitamins A, B1, B2, and C are present in the pointed gourd. Calcium, phosphorus, iron, copper, and potassium are some of the additional minerals it contains. The pointed gourd, according to ayurveda, is a fantastic food for controlling Kapha. The liver is stimulated, and digestion is improved and blood tissue is cleaned.

Review of Literature

The world over, people's diets must include fresh fruits and vegetables. Since they are frequently consumed raw, without sufficient washing or heat treatment, they act as vectors for the spread of dangerous microorganisms linked to human disorders (Biswas *et al.*, 2020). Fruits and vegetables are a vital food group that have been associated with maintaining overall health and a lower prevalence of several chronic diseases due to their high energy content and abundance in minerals, vitamins, fibre, and phenolic compounds (Prasanna *et al.*, 2007). Due to the nutritional content and health benefits of vegetables, there has been a growth in demand for fresh leafy vegetables (FL) and other ready-to-eat (RTE) products over the past 10 years (Losio *et al.*, 2015). FL vegetables are often sold untreated, unclean, and unpackaged, while RTE vegetables are packaged and mildly processed goods intended to be consumed without additional preparation (Losio *et al.*, 2015).

They could also have a variety of molds, like Alternaria. On their surface, Aspergillus and Fusarium are proliferating. Enteric pathogens can infect vegetables. if contaminated water and animal or human excrement are utilised for irrigation and fertiliser, which could result in diseases that affect people (Soriano *et al.*, 2001; Amoah *et al.*, 2009). Important pathogens that have been linked to foodborne diseases linked to various vegetables include *Listeria monocytogenes*, *Salmonella spp.*, *Shigella spp.*, *Clostridium botulinum, Clostridium perfringens*, and *Escherichia coli* (Soriano *et al.*, 2001; Harris *et al.*, 2003; Polcovnicu *et al.*, 2008). Several factors that may contribute to the differences and observed, farm locations, storage temperature or time, and transport condition. The surface bacteria on produce can impact the rate at which food spoils, and may be the source of typical microbes on kitchen surfaces.

Vegetables, which have been seen as a tool for leading healthier lives, have however also frequently been connected to outbreaks of foodborne illnesses (Collejon *et al.*, 2015). According to the available studies, the number of foodborne illnesses caused by tainted produce has grown recently. From 1996 to 2010 in the United States, outbreaks of all foodborne illness accounted for 23% of cases (Algebeleye *et al.*, 2018). Produce was connected to 10% of outbreaks, 35% of hospitalizations, and 46% of fatalities in Europe between 2007 and 2011 (Critzer and Doyle, 2010; Machado Moreira, 2019). The majority of these outbreaks have been linked to vegetables, particularly leafy greens (Harman *et al.*, 2015). As part of the standard procedure for evaluating the microbiological contamination of vegetables, ascertain the levels of total mesophilic aerobic bacteria and gram-negative bacteria of the *Enterobacteriaceae* family, paying particular attention to faecal coliform bacteria as general indicators of pollution. E. coli, Enterobacteriaceae, and coliforms are often used indicators for the assessment of the quality of fresh vegetables and the hygienic conditions present in their production and handling contexts (Szczech *et al.*, 2018; Zheng *et al.*, 2017).

Vegetables and fruits are universally acknowledged as crucial parts of a healthy diet, therefore, many countries, including Canada, have started initiatives to persuade people to eat more of them. Global trade has been impacted by consumer desire for diversity and year-round availability of these items, particularly in countries like Canada where the growing season is short and a large portion of fresh fruits and vegetables are supplied. Since the mid-1990s, there have been increased foodborne illness outbreaks associated to fresh fruit internationally, and actions are being done to address these food safety challenges (Berger *et al.*, 2010; Lynch, Tauxe, and Hedberg, 2009; Sivapalasingam, Friedman, Cohen, and Tauxe, 2004). As a major source of nutrients, vitamins, and fibre, vegetables are crucial to a healthy and balanced diet. In order to stop the spread of many of civilization's ailments, official health agencies in many nations urge the intake of certain foods, especially when they are fresh. In the last few decades, consumption of fresh food has dramatically increased by 30% (Stea *et al.*, 2020).

Bacterial communities can be abundant and diverse in fresh fruits and vegetables. However, most fresh fruit and vegetable research has concentrated on these alone, so we know much less about the diversity and properties of the bacteria's molecules (Leff, 2013). Fresh veggies are seen to be a crucial component of a balanced diet. Such leafy plants are frequently consumed raw or only minimally cooked in order to maintain flavour; however, this approach may increase the risk of contracting foodborne illnesses (Ozlem and Sener, 2005). Pathogenic bacteria can contaminate vegetables during harvest through sewage, untreated irrigation water, surface water, or faeces (Feng *et al.*, 2002). Another cause of contamination is the contaminated water used to rinse veggies and sprinkle them with water to keep them fresh (Froder *et al.*, 2013).

In recent years, human illness epidemics have been linked to using fresh or barely processed fruit consumption Despite their nutritional value, and veggies health advantages (Hedberg *et al.*, 1994; Beuchat, 2002). outbreaks of human illness have been identified as being brought on by tainted produce and Several studies have been published regarding fruit eating. describing how undamaged vegetables get contaminated by bacteria and produce in public markets (Garg *et al.*, 1990). Raw veggies are a source of several potentially contagious pathogenic bacteria that could plant or appear as tiny colonies within the tissues of plants (Beuchat, 2002). The variation of may be impacted by a number of different things. Veggie microbiome, containing typical microorganisms of soil, plant life derived by animal waste, irrigation, or sewage water, transportation, and negligent retail handling. Often, people are exposed to microbial contamination fruit and vegetable handling during harvest or post-harvest processing, as well as to water, dirt, and dust (Carmo *et al.*, 2005; Nguyen, 1994). As a result, many microorganisms, including diseases for plants and people, are present.

Most outbreaks in the Chittagong region have gone unreported due to a lack of surveillance and insufficient screening of these raw veggies, and there is very little information now available in the literature (Nipa *et al.*, 2011). Fresh vegetable consumption has been connected to an increase in the number of incidents of foodborne illness that have been documented in recent years. Beyond all geographical, political, and cultural boundaries, foodborne illness exists. The prevalence of foodborne illnesses continues to negatively impact population health and productivity worldwide, particularly in less industrialized nations (Northo-Clews and Shaw, 2000).

Typically, people eat green chilli and cabbage. uncooked salad ingredients in India and many other countries portions of the globe. Veggies are inexpensive sources of critical vitamins, essential amino acids, and proteins acids (USDA, 2011; Liu, 2013). An example of a part and parcel of Bangladeshi, Indian, Thai, and Mexican-style food. For practically all types of cookery, it is employed. Spices as well as savory foods. Reduced in green chilli while being abundant in fibre, vitamins, and minerals. The chemical capsaicin brings about the green chilli's heat and spiciness. As opposed to that, One of the most often consumed winter vegetables in Bangladesh. It is extensively grown in tropical and subtropical temperate areas of the world having an improvement being produced. According to (Fremaux *et al.*, 2007; Sahilah *et al.*, 2010), pathogens found in contaminated foods may carry virulence genes, toxins, and enzymes that contribute in disease. During processing and distribution, it's possible to bruise raw vegetables, releasing plant components that could serve as potential organic and inorganic substrates for microbes (Zhao *et al.*, 1997; Soriano *et al.*, 2001).

Normally, vegetables from both FL and RTE eaten uncooked. As a result, they may sustain a complex microbial ecology and are well-acknowledged as a possible source of bacterial infections that have led to epidemics all over the world (Denis *et al.*, 2016). In recent years, many scientists' main concerns and areas of attention have shifted to food quality. Additionally, there is a rise in interest in global food safety issues, which will continue to be a major challenge on a global scale (FAO, 2015). (FAO, 2015; Ntuli *et al.*, 2017). The main factors contributing to morbidity are tainted food and water, which are still frequent dangers to public health. Over the past ten years, the number of human diseases brought on by consuming raw vegetables has climbed at an alarming rate (De *et al.*, 2002).

In addition to having nutritional value, fresh produce has a variety of microbiomes that can pass from the stomach to the intestine and develop unique connections with the host, having a variety of effects on human health (Berg *et al.*, 2014). Intriguing links between the gut microbiota and obesity, poor nutrition, cancer, self-motivation, and decision-making have recently been found, and maintaining microbial balance is crucial for maintaining good health (Lozupone *et al.*, 2012). From the time of production until the time of consumption, fruits and vegetables can get contaminated with pathogenic bacteria or rot (Beuchat, 2002; Hassan *et al.*, 2011). Although the majority of their microflora consists of spoilage bacteria, yeasts, fruits and vegetables may include dangerous fungi and molds microorganisms like *Escherichia coli, Bacillus cereus,* and *Salmonella sps., Listeria monocytogenes, Yersinia enterocolitica*, and *Campylobacter spp. clostridium botulinum,* certain viruses, and to genes parasites, too (Beuchat, 2002).

Due to the consumption of contaminated ready-to-eat salad veggies, food borne epidemics are likely to happen everywhere (Ponka *et al*., 1999; Tauxe *et al*., 1997). According to estimates, there are 2.8 million cases of cholera worldwide each year, with 91,000 deaths (Ali *et al*., 2012). Using Hazard Analysis and Critical Control Point (HACCP) principles, it is crucial to identify critical control points to minimise contamination to safe levels (Michalowska and Korczak, 2008). Numerous foodborne illnesses brought on by bacterial pathogens like *Salmonella, Campylobacter, E. coli, Listeria, S. aureus, Klebsiella*, and *Pseudomonas* have steadily risen. These pathogens come into touch with foods during harvest or slaughtering, processing, storage and packaging. Environmental problems have led to the evolution of foodborne bacterial diseases and increased illness risk in the general population (Chibeu, 2013). As a result of eating food that microorganisms have contaminated, foodborne diseases typically manifest as an acute, mild, and self-limiting gastroenteritis with symptoms like nausea, vomiting, and diarrhoea. However, foodborne infections can also lead to a number of chronic diseases that affect the immune, respiratory, cardiovascular, and musculoskeletal systems (Tan *et al*., 2014).

Cabwit *et al.,* (2004). It was researched to evaluate the microbiological standard of fresh, organic vegetables grown in Zambia. Zambian-produced, freshly cut, organic mixed vegetables and green beans were examined for aerobic plate counts, coliforms, *Enterobacteriaceae*, *Escherichia coli*, *Bacillus cereus*, *Clostridium perfringens*, *Listeria monocytogenes*, *Salmonella spp.*, *Staphylococcus aureus*, yeast and mould counts, as well as other contaminants. For the majority of the parameters, the study used 160 samples. The produce was raised on farms intended largely for export. Prior to sampling, the vegetables were treated/washed at the processing facility with a 150lg*mL* chlorine solution. The aerobic plate count varied from 3log10 to 9.7log10 CFU/g, with green beans having the highest level. Vegetable samples fell into the largest category (26.1%) between 3 and 4log10CFU/g. Coliform counts ranged from 1.0 log10 to 1.0. 7.7log10CFU/g, as well. For total coliform levels between 3log10 and 4log10CFU/g, the highest occurrence level was 31.4%. When compared to Enterobacteriaceae counts, which ranged from 1.6log10 to 9.8log10CFU/g with the highest counts reported on green beans, *E. coli* was only detected on mixed vegetables in the range of 0.6 log10 to 3log10CFU/g. For counts in the same range as the aerobic plate counts, the highest incidence level was 25.8%. With an overall range between 1.5log10 and 5.6log10CFU/g, yeast and mould counts revealed the maximum incidence level between 5log10 and 6log10CFU/g. *Salmonella* spp., *L. monocytogenes*, and *S. aureus* were found in 20, 23.1, and 83.9% of samples, respectively. In none of the samples analysed were *C. perfringens* or *B. cereus* found.

Jehra *et al*., (2016). Examined the microbial communities of fresh produce in 105 samples of imported fruits and vegetables, including local samples from Oman, to determine the aerobic plate count and the numbers of Enterobacteriaceae, Enterococcus, and Staphylococcus aureus. The isolated bacteria were identified using molecular (PCR) and biochemical techniques (VITEK 2). In 91% of vegetables and 60% of fruits, enterobacteriaceae were present. 20% of fruits and 42% of vegetables contained enterococcus isolates. From 22 and 7% of the veggies, respectively*, E. coli* and *S. aureus* were recovered. By using VITEK 2 and PCR, 97 bacteria from 21 different species were all successfully identified. The most prevalent species were recognised to be *E. coli, Klebsiella pneumniae, Enterococcus casseliflavus*, and *Enterobacter cloacae* as opportunistic pathogens that could cause concern over how to enhance the fresh produce's microbiological quality. According to phylogenetic trees, the 16S rRNA gene-based clustering of the isolates did not correspond to the origin nations of fresh food. It is possible for opportunistic diseases to go across borders and infect fresh crops, which calls for improved control.

Ayhan *et al.,* (2011). Reported several bacterial pathogens, such as *Shigella* species, *Salmonella* species, *Listeria monocytogenes, E. coli* O157:H7, and *Campylobacte*r, can contaminate vegetables at any point from the field to the point of consumption. It is not surprising that enteric infections can contaminate agricultural produce and lead to outbreaks of sickness after eating given the widespread usage of animal and human faeces in agricultural activity. Listeria monocytogenes can be discovered in rotting vegetables, while spores of *Clostridium perfringens, Clostridium botulinum*, and *Bacillus cereus* isolated from soil free of faecal contamination. **Leclercq *et al.*, (2002)**. Vegetable microbiological examination mainly relies on identifying the presence of indicator organisms. Vegetables contain a significant amount of indicator organisms, albeit they might not all be dangerous. Their discovery shows that humans may have contaminated the vegetables and that potentially harmful organisms may be present. Therefore, the traditional technique for estimating food sanitary quality is based on searching for harmful and indicator bacteria. *Omowaye et al., (2012).* Studied that the vegetable sample analysis, contamination with many parasite species was observed. 15.8% of all samples contained parasites from two different species, while 9.2% of all samples contained parasites from three different species. The presence of many parasites in each sample in this study indicates the potential for vegetable contamination with multiple faeces, which could result in multiple parasitic illnesses in people. It can also be a sign that the local intestinal parasite infection is still present. The handling and consumption of contaminated vegetables present a significant danger to health due to the presence of infectious parasite stages.

Weldezgina and Muleta, (2016). Determined the bacteriological load and safety of various fresh vegetables in Jimma Town, southwest Ethiopia that the Awetu River irrigates. Following established protocols, water and vegetable samples were taken from three distinct irrigation sites and their bacteriological pollutants were examined. The highest overall averages of staphylococci, enterobacteriaceae, aerobic spore formers, aerobic mesophilic bacteria, and total and faecal coliform counts were respectively 10.36 and 716 MPN 100 *mL*-1 and 8.06, 7.10, 6.54, and 2.97 log CFU g1. Vegetable microflora samples were predominately composed of *Bacillus* species (32.7%), *Enterobacteriaceae* (25%) and *Micrococcus* (16%). *Salmonella* spp. and *Staphylococcus aureus* were found in 24.0 and 20.7% of the samples. Ampicillin, cefuroxime sodium, and penicillin G were all ineffective against all of the Staphylococcus aureus isolates (100.0% each). All Ampicillin, cefuroxime sodium, and penicillin G resistance were present in the *Staphylococcus aureus* isolates (100.0% each). Additionally, every Salmonella isolate was resistant to erythromycin, cefuroxime sodium, tetracycline, and penicillin G. (100.0% each).

Swagato *et al.,* (2015). The prevalence and quality of microbes harmful bacteria were discovered in green peppers and cabbage from several stores in Dhaka. A 10 green peppers (Capsicum annuum) and 10 cabbages (Brassica oleracea) were included in the total of 20 samples were gathered at random from 20 sampling locations in the heart of Dhaka. Total coliform and total Staphylococcus aureus were counted among heterotrophic bacteria for each sample using the spread plate and dilution method. The total heterotrophic ranges of bacterial counts (log10cfu/g) were 8.30–11.43 and 10.27–11.83 in samples of cabbage and chilli, respectively. The range of total coliform counts (log10cfu/g) was 5.30 to 8.39 and 6.38 to 8.57 in samples of cabbage and chilli, respectively. The degree of Staphylococcus aureus contamination was less than coliforms and showed counts (log10cfu/g) Regarding cabbage and chilli, the ranges are 4.25 to 7.91 and 5.61 to 7.77, respectively. The presence of Salmonella, Shigella, and Vibrio species was detected following the proper enrichment culture technique. Infected with one or more of the pathogens (Salmonella spp., Vibrio cholerae, or V. parahaemolyticus), more than 40% of both categories of samples were. Conclusion: If consumed raw or with insufficient preparation, green chilli and cabbage constitute a major hazard to the public's health due to high levels of bacterial contamination. Biswas et al., (2020). Isolated and identify the pathogenic bacteria from a variety of widely consumed fresh produce, also known as street food. Seven fresh fruits and vegetables in aseptic condition were procured from several open marketplaces in Chittagong. The bacteria were isolated and counted using selective and non-selective medium. The morphology, biochemical testing (IMViC), and selective differential culture media were used to identify every species. Vibrio spp., Lactobacillus spp., and Pseudomonas spp. were found in the samples and Salmonella spp. took the lead. Fresh fruits and vegetables were all highly coliform and faecal coliform infected (> 1100 CFU/100mL). Guavas ranged in microbial count from 2.1 to 104 CFU/mL, apples from 4.3 to 104 CFU/mL, tomatoes from 8.2 to 104 CFU/mL, cucumbers from 5.0 to 104 CFU/mL, carrots from 1.3 to 104 CFU/mL, and hog plums (Amra) from 7.5 to 104 CFU/mL. These findings showed that the area around Chittagong should be seriously concerned since there may be a significant risk to human health. This suggests the need to raise knowledge of hygienic standards in selling and purchasing fresh fruits and vegetables.

Tambekar and Mundhada, (2006). Reported that the bacterial contamination of salad vegetables was caused by the fact that they are typically eaten raw. Pathogenic microbes can contaminate these veggies during harvest through human handling, harvesting equipment, shipping containers, wild and domestic animals, and more. At the time of intake, pathogens from human and animal reservoirs as well as other environmental pathogens can be discovered and also reported pathogenic bacteria, parasites, and viruses that can infect humans do occasionally coexist with the rotting bacteria, yeasts, and mould that predominate in the microflora on fresh fruits and vegetables. **HemLata and Virupakshaia, (2016)**. Conducted the studies, rotten fruits, vegetables, dairy items, bakery products, poultry products, and spoiled rice were chosen from the local market in the Bagalkot area of Karanataka, India. In particular media like Mannitol Salt Agar, MacConkey Agar, and Cetrimide Agar, collected samples were evaluated. Both morphological and genotypic traits were used to verify samples. Pathogenic bacterial isolates as *Pseudomonas spp* (23.66%), *Staphylococcus aureus* (22.76%), *Salmonella spp.* (21.87%), *E. coli* (22.32%), and *Klebsiella spp.* (8%%) were prevalent in high concentrations. The number of multidrug-resistant isolates (MDR) was significant, and the most common resistant pattern involved the four antibiotic families of cephalosporins, fluoroquinolones, beta-lactams, and aminoglycosides. **Kemajou *et al.*, (2017)**. Analysed that the contains bacteria isolates from water leaves had the highest frequency of infected samples among the vegetable leaf types analysed (16.0%), followed by pumpkin leaves (15.0%), while bitter leaves had the lowest incidence (by far) (13.3%). Escherichia coli (29.3%), Staphylococcus aureus (22.9%), Enterobacter aerogenes (18.3%),

Pseudomonas aeruginosa (8.2%), Shigella species (5.5%), Alcaligenes feacalis (4.6%), Micrococcus species (3.7%), and Salmonella species were the bacteria species that were isolated

(2.1%). Ten different bacterial species were present in all samples of water leaves, pumpkin leaves, and green leaves. Escherichia coli, Staphylococcus aureus, Enterobacter aerogenes, and Pseudomonas aeruginosa were most frequently found in water leaves (39.6, 16.8, and 11.4%, respectively), followed by pumpkin leaves (29.7, 18.1, and 11.4%, respectively 12.9%). Salmonella species, Pseudomonas aeruginosa, Citrobacter species, Micrococcus species, and other isolates could not be made from samples of bitter leaves. Gram negative bacteria were extremely sensitive to ofloxacin and ciprofloxacin (55.6-100%) and less sensitive to tetracycline (0.0-22.7%), according to the results of an analysis of antibiotic susceptibility. Bacillus cereus exhibited the lowest susceptibility to erythromycin (9.1%), while gram-positive bacteria had a susceptibility to pefloxacin (90.9-100%), ciprofloxacin (81.8-100%), and erythromycin. **Farahnaaz and Jessica, (2013)**. determining the frequency of microorganisms among them, it may be possible to assess if freshly cut vegetables can serve as substrates to encourage the production of the deteriorating micro flora. On the basis of these hypotheses, the current study sought to investigate the likelihood of deteriorating microflora and pathogens, such as E. coli, Staphylococcus spp., Vibrio spp., Klebsiella spp., Salmonella spp., Shigella spp., Pseudomonas spp., Aeromonas spp., and Listeria spp., among the frequently Samples of carrot, cucumber, and tomato were collected from various hotels and restaurants and cleaned to remove pollutants. reduction of *Salmonella spp., Shigella spp., Pseudomonas spp., and Listeria spp.* in carrot and tomato samples, as well as *Shigella, Pseudomonas* spp., and cucumber samples.

World Health Organisation, (2016). Stated that the vegetables have health promoting characteristics, being a source of vitamins and minerals, and phytochemical some of which are antioxidant, phytoestrogens and anti-inflammatory agents. The recent increase in awareness of the health benefits of vegetables has resulted in increased consumption. Insufficient fruit and vegetable consumption contributes to poor health and increases the risk of non communicable diseases.

Aims and Objective

The main aim of the present study was to Isolation and Enumeration of Bacteria from Common Green Vegetables Near in the Market (Maya Bazaar) of Ayodhya District, Uttar Pradesh.

- Isolation of Bacteria from selected common green vegetables (tomatoes, cabbages, capsicum and pointed gourd) near in the Market.
- Morphological Identification of Isolated Bacteria from common green vegetables with the help of Light Microscope.

Materials and Methods

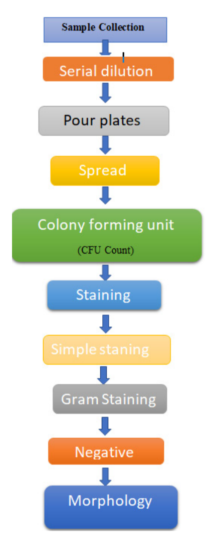
Study Area

Ayodhya district is one of the 75 districts of Uttar Pradesh state in Northern India lies between 26°46^{1} to 45°81^{1}N and 82°14^{1} to37°26^{1}E . The district has been divided into eleven blocks: Mawai, Rudauli, Sohawal, Masodha, Pura bazaar, Maya bazaar, Amaniganj, Milkipur, Haringtonganj,

Bikapur and Tarun. The district extends over an area of 2,764 km^{2}. Ayodhya (Faizabad) district (Fig. 16) has a population of 2,468,371 in 2011 which is equal to the population of Kuwait and Nevada state of USA. Ayodhya district is the administrative headquarter of Faizabad division.

Sampling and Sample Collection

A total of 4 raw vegetable samples such as tomato, capsicum, cabbage and pointed gourd were collected from different site of study area (Maya bazaar) of Ayodhya District within a time from of March, 2022 to June, 2022. All samples were collected in sterile polythene bag in an insulated box with ice to maintain a temperature ranging from 4 to 6°C and brought in the Research Laboratory of Microbiology, Centre of Excellence, (DST-FIST SUPPORTED), Department of Microbiology, Dr. Rammanohar Lohia Avadh University, Ayodhya (Faizabad), for analyzed the sample within one hour after procurement. Samples were collected early in



Flow chart 1: Study design

the morning and transported to the laboratory as soon as possible, according to the method suggested by American Public Health Association (APHA).

Processing of Raw Vegetable Samples

Prior to introducing the test of bacteria into the vegetables, all 4 samples (tomatoes, cabbage, capsicum, pointed gourd) were washed with distilled water in petridish and then 1-1 *mL* of the washing sample add in each serial dilution of twelve test tube and spreading with the help of spreader on petriplate of culture media then spreading culture media plate setup inside cooling BOD incubator at 36 hours after remove from the incubator then proceed for slide preparation.

Preparation of Nutrient Agar Media

Nutrient Agar Medium (NAM) is a general purpose complex medium used for the cultivation of microbes supporting growth of a wide range of non-fastidious organisms. Different types of bacteria can grow in NAM, and it contains nutrients required for the growth of microorganisms.

Composition of Nutrient Agar Media

- 0.5% w/v, Peptone (it is an enzymatic digest of animal protein. Peptone is the principal source of organic nitrogen for the growing bacteria)
- 0.3% w/v, beef extract (provide vitamins, carbohydrates, organic nitrogen compounds and salts).
- 1.5% w/v, agar (it is the solidifying agent)
- 0.5% NaCl (maintains osmolarilty in the medium)
- Distilled water
- pH is adjusted to neutral (7±0.2)

Methods

- Suspend each constituent in 800 *mL* distilled water, followed by adjustment of pH to 7.0 using
- 0.1 N NaOH /0.1 N HCl.
- Add agar in to the medium and adjust the final volume to 1 litre using distilled water.
- Heat this mixture while stirring to bring the agar in to molten state.

Figure 1: Sample collection and processing for isolation of bacteria from green vegetables.

- Autoclave the dissolved mixture at 121°C and 15 lb/inch2 pressure for 15 minutes.
- Once the nutrient agar has been autoclaved, allow it to cool but not solidify.
- Pour the sterilized nutrient agar medium into the sterile Petri dishes.

Isolation of bacteria from raw vegetables

Robert Koch developed pure culture isolation technique. Normally microbial population exists as a mixture of many other cell types, this laboratory technique is used to produce pure culture from these population. This method also counts the number of viable organisms in a liquid such as water, milk, broth culture. The main purpose of the pour plate method is to isolate the pure culture from a mixture of different populations and demonstrate the cultural characteristics of the bacteria such as color, texture, size, elevation etc. Serial dilutions of the inoculum (serially diluting the primary specimen) are added to the sterile petri dishes followed by molten and cooled (42–45°C) agar medium. The inoculums and medium were completely mixed by revolving the plates, which are then left to solidify. The inoculums may also be spread on solidified agar medium in sterile Petri dishes using sterile glass or plastic spreader. After 24 hours incubation of inoculated Petri dishes at an appropriate temperature are observed for the appearance of individually isolated colonies growing everywhere in the medium. The pure colonies which are of varying size, shape and colour may be isolated/transported into test tube culture media to prepare pure cultures (Figure 1).

Streak Plate method for isolation of pure culture

The isolated bacterial colonies may be a kind of mix culture. For the purification of bacterial cultures from the previous Petri dishes, the isolated bacterial colony may be purified following streak plate method.

Total number of colony-forming units (CFU) on the surface of an agar medium is enumerated. The Calculation of CFU/mL is done by using the formula:

$$\text{Total viable counts (CFU/mL)} = \frac{\text{CFU x dilution factor}}{\text{wt or volume of sample}}$$

Staining Techniques

Bacteria are practically semitransparent and difficult to be seen in the unstained form. The bacteria are stained to make them more clearly visible (to develop contrast between bacterial cells and background). Dyes are the salts containing an organic and an inorganic ion. The salts are composed of positively charged ions (cations) and negatively charged ions (anions). Depending on its charge, the dye base is either associated with an anion or a cation, it is called a basic dye. A coloring agent is used for general purpose (e.g. textile coloring) is called as a dye; the one which is used for colouring biological materials is referred to as a stain.

Staining process are mainly two types:

1. Gram Staining 2. Negative Staining/Indirect Staining

Gram's Staining

This is one of the most useful staining techniques in the identification of bacteria. In 1884, Danish bacteriologist Christian gram developed this staining technique that classified all cell walled bacteria into two groups: gram-positive and gram-negative. The Gram stain is a differential stain requiring a primary stain and a counter stain. The primary stain is crystal violet, which is followed by treatment with iodine solution. The iodine is a mordant that combines with primary stain to form an insoluble coloured compound called as crystal violet-iodine (CVI) complex. The function of a mordant is to increase affinity between dye-iodine complex and the bacterial cell. After decolorizing, usually with 95% ethanol or acetone, a counter stain safranine (secondary stain) is applied to smear (thin uniform film of bacteria over slide). The procedure is based on the ability of microorganisms to retain the purple colour of crystal violet during decolorization with alcohol/acetone. Bacteria that resist decolorization and retain the crystal violet-iodine complex appear purple or deep blue microscopically and are classified as gram-positive. Conversely, cells that loose the colour of crystal violet-iodine complex during decolorization step and later accept the safranine counterstain and appear red, are referred to as gram-negative.

Materials

Microscope, glass slide, Bunsen burner, sprit lamp, wash bottle, basic stains; crystal violet, safranine, bacterial culture, inoclution loop.

Procedure

- Take a clean, greas free slide. Using inoculation loop, transfer a loopful of the culture (if broth culture) in the centre of glass slide. However, if the culture is taken from solid medium, first put a drop of water on the glass slide and then aseptically transfer the culture employing inoculation loop.
- Make a thin film (smear) by spreading the culture with the help of inoculation loop. The smear should be neither too thick nor too thin
- Air dry the smear, heat fix the smear by passing the slide three-four times through the flame. The aim of fixation is to kill the bacteria and to fix it to the glass slide.
- Pour few drops of crystal violet and allow to react for 60 seconds, wash off the smear gently under the tap water so as to remove the excess stain, then pour grams iodine for 60 second, wash off, after washing add 95% ethanol for 10 sec and wash off and finally pour safranine for 60 seconds and wash off,
- Air-dry very carefully.
- Examine the preparation directly under oil immersion objective.

Negative or Indirect Staining

In such type of staining technique, the bacteria are not stained directly rather the stains gets deposited surrounding the bacterial cells and hence the name negative or indirect staining. This technique is performed employing acidic stains such as eosin, nigrosine, etc. This technique can be useful for determining cell morphology and size.

Materials

Microscope, glass slide, acidic stain (nigrosine black), inoculation loop, broth or agar culture.

Procedure

- Put a drop of bacterial culture at one end of the glass slide and add a drop of acidic stain to it.
- Mix the contents with inoculating loop and make a thin and uniform film employing another glass slide
- Allow the smear to air dry.
- Examine the slide under oil immersion objective.

Results

The objective of the current investigation was to identify and enumeration of bacteria common green vegetables. Gram positive and gram negative bacteria has been isolated and identified from collected samples via enrichment, selective plating, gram staining methods and morphological based approaches. Following that, phenotypic characteristics of the verified isolates were determined.

In the present study, four different types of green raw vegetables (Capsicum, Pointed gourd, Tomatoes and Cabbages*)* collected from the local market of Ayodhya and isolated bacteria from their and identified on the basis of morphological analysis, the identified bacteria as like *Coccus* isolated from three raw green vegetables (Pointed gourd, Tomatoes and Cabbages) but not identified bacteria of *capsicum* on the basis of morphological character and the isolated bacteria are showing in the (Figures 2 and 3) and colony forming units and colony counting showing in the (Tables 1 and 2).

Discussion

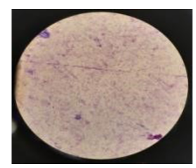
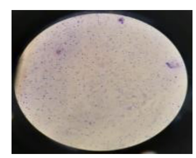
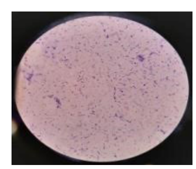
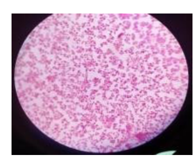
The purpose of this study was to present the isolated, identification, and analysis of data from surveys conducted over a four-month period (March 2022 to June 2022) looking into the bacterial contamination of a variety of fresh raw green vegetables available at local retail markets in Ayodhya, Uttar Pradesh, including leafy vegetables, leafy herbs, green onions, tomatoes, cantaloupes, and berries. The analysis's goals were to produce a baseline to guide decisions about food safety, as well as a summary of the findings and trends seen.

Since it is widely accepted that fruits and vegetables are essential components of a healthy diet, several nations,

Table 1: Total number of colony-forming units (CFU) on the surface of an agar medium is enumerated and Calculation of CFU/mL.

S. No.	*Vegetables*	*Dilution Factor*	*No. of Colonies plates I, II*	*Average No. of Colonies plates*	*CFU/mL* $= \frac{\text{CFU x dilution factor}}{\text{wt or volume of sample}}$
I	*Capsicum*	10-4	76	$\frac{76+62}{2} = 69$	$\frac{76\times4}{0.1} = 3.04x10^2$
II	*Capsicum*	10-6	62		$\frac{62\times4}{0.1} = 2.48x10^4$
I	*Pointed gourd*	10-4	64	$\frac{64+43}{2} = 53.5$	$\frac{64\times4}{0.1} = 2.56x10^2$
II	*Pointed gourd*	10-6	43		$\frac{43\times4}{0.1} = 1.72x10^4$
I	*Tomatoes*	10-4	94	$\frac{94+76}{2} = 85$	$\frac{94\times4}{0.1} = 3.76x10^2$
II	*Tomatoes*	10-6	76		$\frac{76\times4}{0.1} = 3.04x10^4$
I	*Cabbages*	10-4	107	$\frac{107+46}{2} = 76.5$	$\frac{107\times4}{0.1} = 4.28x10^2$
II	*Cabbages*	10-6	46		$\frac{46\times4}{0.1} = 1.84x10^4$

Table 2: Characteristic features of isolated bacteria from raw green vegetables

S. No.	*Vegetables*	*Staining*	*Shape*	*colour*	*Isolated Bacteria*
1.	*Capsicum*	Gram Staining Negative Staining Simple Staining	Rod shape Small Rod Rod shape	Purple White Purple	
2.	*Pointed gourd*	Gram Staining Negative Staining Simple Staining	Coccus Rod Shape Rod Shape	Pink White Purple	
3.	*Tomatoes*	Gram Staining Negative Staining Simple Staining	Small Rod Coccus Cocuus	Pink White Purple	
4.	*Cabbages*	Gram Staining Negative Staining Simple Staining	Long Rod Coccus Coccus	Pink White Purple	

including Canada, have launched campaigns to encourage people to consume more of them. Consumer demand for variety and year-round availability of these goods has had an impact on international trade, particularly in nations like Canada where the growing season is short and a substantial amount of fresh fruits and vegetables are provided. International occurrences of foodborne illness linked to fresh fruit have increased since the mid-1990s, and measures are being taken to address these issues with food safety (Berger *et al.*, 2010; Lynch, Tauxe, and Hedberg, 2009; Sivapalasingam, Friedman, Cohen, and Tauxe, 2004).

In our investigation, it was discovered that tomato samples encouraged the growth of nearly all of the test bacteria, carrot samples encouraged the growth of all bacteria except *Shigella* spp., and cucumber samples encouraged the growth of all bacteria except *Pseudomonas*. Notably, samples of carrot, tomato, and cucumber showed no difference in the growth of *Pseudomonas* spp. or

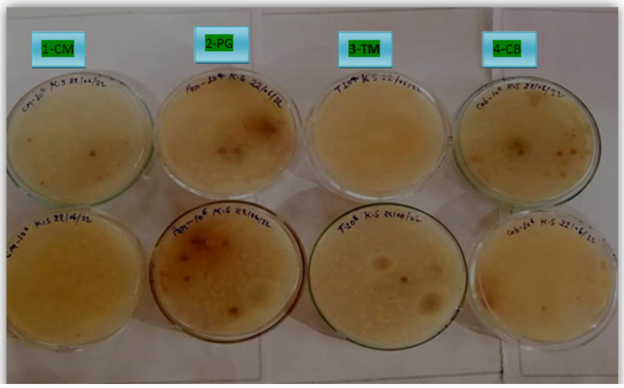


Figure 2: Showing four different bacteria culture from common green vegetables: 1-CM (Capsicum), 2-PG (Pointed gourd), 3-TM (Tomato), and 4-CB (Cabbage).

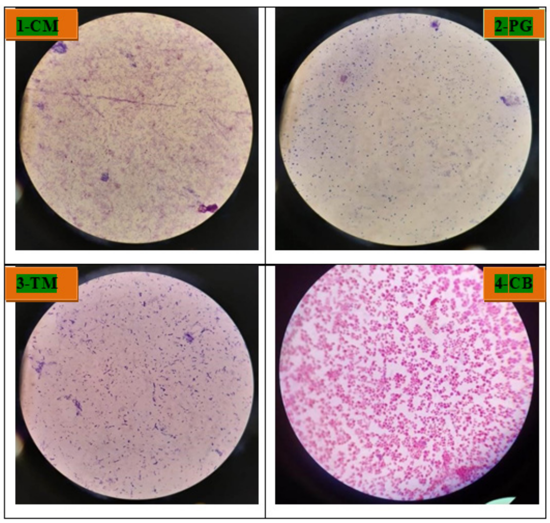


Figure 3: Observe the isolated bacteria under the Light microscope for morphological identification.

Salmonella spp. *Staphylococcus* spp. and *E. coli* demonstrated the lowest growth rates among the pathogens examined, designating them as the pathogens with the most possibility for survival. Given that infections are ubiquitous on most surfaces and that they are part of the body's regular flora, this fact can be concerning. It may also be a sign of strong environmental conditions that encourage the growth of organisms, which can readily be transmitted via faeces contamination and skin contact.

The prevalence of bacterial contamination was generally quite low in the four commodities that were the subject of this study. Rarely were bacterial pathogens found and isolated. *Shigella* (23,286 samples analysed), *E. coli* O157 (23,805 samples studied), and *Campylobacter* were the three pathogens that could not be found in any of the samples tested (8866 samples analysed). Because of this, it was discovered that the calculated prevalence intervals for these pathogens in the various produce groups studied were as low as (0, 0.03%) for *E. coli* O157 in leafy vegetables, the group with the largest sample size, and as high as (0, 0.28%) for *Shigella* in berries, the group with the smallest sample size. Similar to this, just ten of the 29,391 samples examined yielded

Salmonella detection and isolation. These favourable samples included found in cantaloupes, fresh leafy herbs, green onions and leafy vegetables. Salmonella's low incidence in fresh produce was corroborated by the prevalence figures. Leafy herbs and cantaloupes had the greatest positive rates, with prevalence ranges of (0.04, 0.29%) and (0.02, 0.23%), respectively.

Leafy herbs' bacterial contamination seems to fluctuate over time, but no obvious seasonal pattern could be seen. In general, leafy herbs were the most polluted of the commodity groups included in these surveys, and the statistics tend to suggest that this pattern is fairly stable throughout the seasons. In the other commodity categories under study, detection of bacterial pathogens and common *E. coli* (at levels above 100 CFU or MPN/g) was so seldom that, not unexpectedly, no particularly noteworthy events in bacterial contamination arose. Given that tomatoes have been linked to numerous significant Salmonella outbreaks in North America (Hanning *et al.*, 2009), it is incredibly surprising that none of the 4837 samples gathered over three years of surveillance showed any contamination with the pathogen bacteria.

Fresh produce is a significant source of vitamins, minerals, and fibre, which has led to an increase in consumption over the past two decades (Olaimat and Holley, 2012). Additionally, consumers are increasingly concerned with maintaining healthy eating habits and are more aware of the health benefits of fresh fruits and vegetables. However, instances of foodborne illness linked to fresh vegetables have grown concurrently (Warriner *et al.*, 2009). Raw vegetables can contain a variety of potentially infectious pathogenic bacteria that can grow on plants or appear as small colonies inside plant tissues (Beuchat, 2002). Numerous factors may have an impact on the variation of microbiome of vegetables, which includes regular soil microbes, plant life generated from animal waste, irrigation, or sewage water, transportation, and careless retail handling. During harvest, sewage, untreated irrigation water, surface water, or faeces can contaminate vegetables with dangerous germs (Feng *et al.*, 2002). The polluted water used to rinse vegetables and mist them with water to keep them fresh is another source of infection (Froder *et al.*, 2013).

Conclusion

The level of microbial contamination in fresh raw vegetables has been the subject of numerous research in the past, but it is still unknown how long the infection will last. The investigation showed the presence of many isolated bacteria on raw green vegetables in close proximity to the market in Ayodhya, Uttar Pradesh, India. In order to identify rising developing globe, intensive isolation surveillance is required. As further heat treatment is necessary for the preparation of vegetables, it is crucial to properly wash the vegetables and soak them in food-grade antibacterial chemicals for a sufficient amount of time to kill any infections and drastically lower the microbial burden.

The bacterial contamination of green, raw vegetables was discovered in this investigation (Capsicum, Tomatoes, Pointed gourd and Cabbages). *Coccus sps*. has been morphologically identified in that way based on a distinguishing feature. After Gram positive and Gram negative staining, it is confirmed. On the basis of morphological characteristics and the isolated bacteria, the identified bacteria as resembling *Coccus* isolated from three raw green vegetables (pointed gourd, tomatoes, and cabbages) but not capsicum.

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